EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	30569	chaperon$ or aggregat$ near4 (inhibit$ or suppress$ or decreas$ or prevent$)	US-PGPUB; USPAT	ADJ	OFF	2007/07/13 16:20
L2	345	1 near10 fusion	US-PGPUB; USPAT	ADJ	OFF	2007/07/13 16:20
L3	94	1 same (archae$ or methanococcus or thermococcus or methanosarcina)	US-PGPUB; USPAT	ADJ	OFF	2007/07/13 16:22
L4	7	2 and 3	US-PGPUB; USPAT	ADJ	OFF	2007/07/13 16:22

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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 17:00:34 ON 13 JUL 2007

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 17:00:46 ON 13 JUL 2007
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s chaparon? or aggregat?(5a)(inhibit? or suppress? or prevent? or decreas?)
FILE 'MEDLINE'
             6 CHAPARON?
        126941 AGGREGAT?
       1351325 INHIBIT?
        313488 SUPPRESS?
       1095737 PREVENT?
       1100277 DECREAS?
         27826 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L1       27832 CHAPARON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'SCISEARCH'
             8 CHAPARON?
        162666 AGGREGAT?
       1135992 INHIBIT?
        337809 SUPPRESS?
        473715 PREVENT?
       1148607 DECREAS?
         14087 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L2       14094 CHAPARON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'LIFESCI'
             9 CHAPARON?
         32220 AGGREGAT?
        372382 INHIBIT?
        101581 SUPPRESS?
        103134 PREVENT?
        267178 DECREAS?
          2908 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L3        2917 CHAPARON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOTECHDS'
             0 CHAPARON?
          4562 AGGREGAT?
         64314 INHIBIT?
         11382 SUPPRESS?
         31700 PREVENT?
         28746 DECREAS?
           674 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L4         674 CHAPARON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOSIS'
            15 CHAPARON?
        139518 AGGREGAT?
       1546560 INHIBIT?
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         342301 SUPPRESS?
         536430 PREVENT?
        1325160 DECREAS?
          18201 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L5        18216 CHAPARON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
                DECREAS?)

<------User Break------>

SEARCH ENDED BY USER

=> del his
DELETE ALL L# ITEMS? (Y)/N:y

=> s chaperon? or aggregat?(5a)(inhibit? or suppress? or prevent? or decreas?)
FILE 'MEDLINE'
          17547 CHAPERON?
         126941 AGGREGAT?
        1351325 INHIBIT?
         313488 SUPPRESS?
        1095737 PREVENT?
        1100277 DECREAS?
          27826 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L1        44552 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
                DECREAS?)

FILE 'SCISEARCH'
          18029 CHAPERON?
         162666 AGGREGAT?
        1135992 INHIBIT?
         337809 SUPPRESS?
         473715 PREVENT?
        1148607 DECREAS?
          14087 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L2        31230 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
                DECREAS?)

FILE 'LIFESCI'
           6085 CHAPERON?
          32220 AGGREGAT?
         372382 INHIBIT?
         101581 SUPPRESS?
         103134 PREVENT?
         267178 DECREAS?
           2908 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L3         8674 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
                DECREAS?)

FILE 'BIOTECHDS'
            811 CHAPERON?
           4562 AGGREGAT?
          64314 INHIBIT?
          11382 SUPPRESS?
          31700 PREVENT?
          28746 DECREAS?
            674 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L4         1451 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
                DECREAS?)

FILE 'BIOSIS'
          15189 CHAPERON?
         139518 AGGREGAT?
        1546560 INHIBIT?
         342301 SUPPRESS?
         536430 PREVENT?
```

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       1325160 DECREAS?
         18201 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L5       32565 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'EMBASE'
         13054 CHAPERON?
        106152 AGGREGAT?
       1238963 INHIBIT?
        289842 SUPPRESS?
        848535 PREVENT?
       1025425 DECREAS?
         18343 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L6       30672 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'HCAPLUS'
         17608 CHAPERON?
        242070 AGGREGAT?
       1941959 INHIBIT?
        424017 SUPPRESS?
        953241 PREVENT?
       2394543 DECREAS?
         33228 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L7       49870 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'NTIS'
           110 CHAPERON?
         13355 AGGREGAT?
         21951 INHIBIT?
         15207 SUPPRESS?
         53259 PREVENT?
         53650 DECREAS?
           167 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L8           274 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'ESBIOBASE'
         10592 CHAPERON?
         42748 AGGREGAT?
        519843 INHIBIT?
        139656 SUPPRESS?
        171821 PREVENT?
        428879 DECREAS?
          5469 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L9       15422 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOTECHNO'
          5718 CHAPERON?
         22679 AGGREGAT?
        301415 INHIBIT?
         79558 SUPPRESS?
         71195 PREVENT?
        171676 DECREAS?
          3551 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L10       8911 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'WPIDS'
           619 CHAPERON?
         65771 AGGREGAT?
        279324 INHIBIT?
        247088 SUPPRESS?
```

```
       1830416 PREVENT?
        273857 DECREAS?
          7902 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L11       8491 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

TOTAL FOR ALL FILES
L12     232112 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

=> s l12(10a)fusion
FILE 'MEDLINE'
        148477 FUSION
L13        106 L1 (10A)FUSION

FILE 'SCISEARCH'
        134295 FUSION
L14        107 L2 (10A)FUSION

FILE 'LIFESCI'
         42007 FUSION
L15         68 L3 (10A)FUSION

FILE 'BIOTECHDS'
         26537 FUSION
L16         67 L4 (10A)FUSION

FILE 'BIOSIS'
        108808 FUSION
L17        110 L5 (10A)FUSION

FILE 'EMBASE'
         86966 FUSION
L18         93 L6 (10A)FUSION

FILE 'HCAPLUS'
        272840 FUSION
L19        275 L7 (10A)FUSION

FILE 'NTIS'
         23053 FUSION
L20          0 L8 (10A)FUSION

FILE 'ESBIOBASE'
         45290 FUSION
L21         85 L9 (10A)FUSION

FILE 'BIOTECHNO'
         42345 FUSION
L22         61 L10(10A)FUSION

FILE 'WPIDS'
         55379 FUSION
L23         68 L11(10A)FUSION

TOTAL FOR ALL FILES
L24       1040 L12(10A) FUSION

=> s l12(10a)(archae? or methanococcus or thermococcus or methanosarcina)
FILE 'MEDLINE'
         12607 ARCHAE?
          1025 METHANOCOCCUS
           424 THERMOCOCCUS
           760 METHANOSARCINA
L25        128 L1 (10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
```

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               INA)

FILE 'SCISEARCH'
         22633 ARCHAE?
          1351 METHANOCOCCUS
           640 THERMOCOCCUS
          1407 METHANOSARCINA
L26        149 L2 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'LIFESCI'
          8018 ARCHAE?
           821 METHANOCOCCUS
           342 THERMOCOCCUS
           835 METHANOSARCINA
L27         88 L3 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'BIOTECHDS'
          1693 ARCHAE?
           237 METHANOCOCCUS
           233 THERMOCOCCUS
           418 METHANOSARCINA
L28         28 L4 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'BIOSIS'
         30872 ARCHAE?
          1588 METHANOCOCCUS
           595 THERMOCOCCUS
          1570 METHANOSARCINA
L29        137 L5 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'EMBASE'
          9532 ARCHAE?
           990 METHANOCOCCUS
           397 THERMOCOCCUS
           924 METHANOSARCINA
L30        111 L6 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'HCAPLUS'
         26182 ARCHAE?
          1575 METHANOCOCCUS
           779 THERMOCOCCUS
          1565 METHANOSARCINA
L31        212 L7 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'NTIS'
          4795 ARCHAE?
            36 METHANOCOCCUS
             3 THERMOCOCCUS
            49 METHANOSARCINA
L32          4 L8 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'ESBIOBASE'
         10292 ARCHAE?
           655 METHANOCOCCUS
           355 THERMOCOCCUS
           553 METHANOSARCINA
L33        113 L9 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)
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FILE 'BIOTECHNO'
          5361 ARCHAE?
           719 METHANOCOCCUS
           284 THERMOCOCCUS
           662 METHANOSARCINA
L34         78 L10(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'WPIDS'
           670 ARCHAE?
           104 METHANOCOCCUS
           151 THERMOCOCCUS
            83 METHANOSARCINA
L35         22 L11(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

TOTAL FOR ALL FILES
L36       1070 L12(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

=> s l24 and l36
FILE 'MEDLINE'
L37          0 L13 AND L25

FILE 'SCISEARCH'
L38          0 L14 AND L26

FILE 'LIFESCI'
L39          0 L15 AND L27

FILE 'BIOTECHDS'
L40          4 L16 AND L28

FILE 'BIOSIS'
L41          0 L17 AND L29

FILE 'EMBASE'
L42          0 L18 AND L30

FILE 'HCAPLUS'
L43         12 L19 AND L31

FILE 'NTIS'
L44          0 L20 AND L32

FILE 'ESBIOBASE'
L45          0 L21 AND L33

FILE 'BIOTECHNO'
L46          0 L22 AND L34

FILE 'WPIDS'
L47          4 L23 AND L35

TOTAL FOR ALL FILES
L48         20 L24 AND L36

=> dup rem l48
PROCESSING COMPLETED FOR L48
L49          14 DUP REM L48 (6 DUPLICATES REMOVED)

=> d tot

L49  ANSWER 1 OF 14  HCAPLUS  COPYRIGHT 2007 ACS on STN
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TI Versatile platform for nanotechnology based on circular permutations of chaperonin TF55β from Sulfolobus shibatae
SO U.S. Pat. Appl. Publ., 151 pp., Cont.-in-part of U.S. Ser. No. 494,853.
CODEN: USXXCO
IN Paavola, Chad D.; Trent, Jonathan D.; Chan, Suzanne L.; Li, Yi-Fen; McMillan, R. Andrew; Kagawa, Hiromi
AN 2006:367306 HCAPLUS
DN 144:407655

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2006084792	A1	20060420	US 2005-194991	20050801
WO 2003080796	A2	20031002	WO 2002-US35889	20021108
WO 2003080796	A9	20040429		
WO 2003080796	A3	20051222		

PI
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2005130258	A1	20050616	US 2004-494853	20040506

L49 ANSWER 2 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Use of chaperonin PPIase for enhancement of poorly expressed proteins and immobilization of the proteins for drug screening
SO Jpn. Kokai Tokkyo Koho, 41 pp.
CODEN: JKXXAF
IN Ideno, Akira; Furuya, Masahiro
AN 2005:54187 HCAPLUS
DN 142:149781

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2005013067	A	20050120	JP 2003-181394	20030625

PI

L49 ANSWER 3 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI An engineered chaperonin caging a guest protein: structural insights and potential as a protein expression tool
SO Protein Science (2005), 14(2), 341-350
CODEN: PRCIEI; ISSN: 0961-8368
AU Furutani, Masahiro; Hata, Jun-Ichi; Shomura, Yasuhito; Itami, Keisuke; Yoshida, Takao; Izumoto, Yoshitaka; Togi, Akiko; Ideno, Akira; Yasunaga, Takuo; Miki, Kunio; Maruyama, Tadashi
AN 2005:117819 HCAPLUS
DN 142:349731

L49 ANSWER 4 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Protein expression assisted by archaeal molecular chaperones and application views
SO Baiosaiensu to Indasutori (2005), 63(4), 237-240
CODEN: BIDSE6; ISSN: 0914-8981
AU Furutani, Masahiro; Ideno, Akira
AN 2005:417273 HCAPLUS
DN 143:21474

L49 ANSWER 5 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI Novel immunogen comprising fused protein of full-length or part of antigen (serotonin 5 HT1aR) and folding factor (peptidylprolyl isomerase) or its subunit bonded together through peptide bonds, useful for inducing immune response;
vector-mediated immunogen gene transfer and expression in host cell for recombinant vaccine

AU IZUMOTO Y; HATA J; IDENO A; FURUTANI M
AN 2004-25618 BIOTECHDS
PI WO 2004092221 28 Oct 2004

L49 ANSWER 6 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI Producing recombinant antibody from fusion protein having heavy chain and/or light chain antibody connected to chaperonin through peptide bond, involves expressing fusion protein and producing antibody by cleaving fusion protein;
recombinant antibody production via plasmid expression in host cell
AN 2004-13126 BIOTECHDS
PI JP 2004081199 18 Mar 2004

L49 ANSWER 7 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI Sustained-release formulation useful for sustained release of bioactive protein, comprises fusion protein consisting of bioactive protein or low molecular medical agent coupled to conveyance protein and chaperonin;
recombinant protein production via plasmid expression in host cell for use in protein release
AN 2004-10167 BIOTECHDS
PI JP 2004026714 29 Jan 2004

L49 ANSWER 8 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI Novel fusion protein comprising chaperonin and protein having enzyme activity connected through peptide bond, useful as immobilized enzyme bioreactor;
involving vector-mediated gene transfer and expression in host cell
AN 2004-10388 BIOTECHDS
PI JP 2004026713 29 Jan 2004

L49 ANSWER 9 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Chaperonin-target protein complex, method of producing the same, method of stabilizing target protein, method of immobilizing target protein, method of analyzing the structure of target protein, sustained-release preparation and method of producing antibody against target protein
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Ideno, Akira; Hata, Jun-ichi; Togi, Akiko; Furutani, Masahiro
AN 2004:965292 HCAPLUS
DN 141:428008

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2004096859	A1	20041111	WO 2004-JP6189	20040428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2004234282	A1	20041111	AU 2004-234282	20040428
CA 2522263	A1	20041111	CA 2004-2522263	20040428
EP 1619208	A1	20060125	EP 2004-730054	20040428
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, HR				
US 2007059794	A1	20070315	US 2005-554747	20051028

L49 ANSWER 10 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Increase the efficiency of fusion protein production by using chaperonin
SO Jpn. Kokai Tokkyo Koho, 13 pp.

CODEN: JKXXAF
IN Hata, Junichi; Furuya, Masahiro
AN 2004:986000 HCAPLUS
DN 141:420999

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2004321141	A	20041118	JP 2003-124353	20030428

L49 ANSWER 11 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Expression of fusion protein containing chaperonin to enhance the efficiency of target protein expression
SO Jpn. Kokai Tokkyo Koho, 23 pp.
CODEN: JKXXAF
IN Ideno, Akira; Hata, Junichi; Togi, Akiko; Furuya, Masahiro
AN 2004:71225 HCAPLUS
DN 140:140647

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2004024095	A	20040129	JP 2002-184787	20020625

L49 ANSWER 12 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Expression vector containing chaperonin PPIase for improvement of the expression efficiency for foreign proteins
SO PCT Int. Appl., 73 pp.
CODEN: PIXXD2
IN Ideno, Akira; Maruyama, Tadashi; Furutani, Masahiro
AN 2004:3039 HCAPLUS
DN 140:72133

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2004001041	A1	20031231	WO 2003-JP8020	20030625
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
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CA 2490384	A1	20031231	CA 2003-2490384	20030625
AU 2003243969	A1	20040106	AU 2003-243969	20030625
EP 1516928	A1	20050323	EP 2003-733573	20030625
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 2005130259	A1	20050616	US 2003-511098	20030625

L49 ANSWER 13 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Recombinant protein expression as chaperonin fusion protein for X-ray crystal structure analysis
SO Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
IN Furuya, Masahiro; Hata, Junichi
AN 2003:734803 HCAPLUS
DN 139:257016

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2003261597	A	20030919	JP 2002-353990	20021205

L49 ANSWER 14 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
TI Preparation of recombinant protein as chaperon fusion protein
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2

IN Furutani, Masahiro; Hata, Junichi; Togi, Akiko
AN 2002:504945 HCAPLUS
DN 137:62163

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002052029	A1	20020704	WO 2001-JP11438	20011226
	WO 2002052029	A9	20030424		
	W: AU, CA, JP, KR, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	CA 2432716	A1	20020704	CA 2001-2432716	20011226
	AU 2002217505	A1	20020708	AU 2002-217505	20011226
	EP 1354959	A1	20031022	EP 2001-272326	20011226
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR				
	US 2004146969	A1	20040729	US 2003-451883	20031016

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L49 ANSWER 1 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB The present invention provides chaperonin polypeptides which are modified to include N-terminal and C-terminal ends that are relocated from the central pore region to various different positions in the polypeptide which are located on the exterior of the folded modified chaperonin variant. In the modified chaperonin, the naturally-occurring N-terminal and C-terminal ends are joined together directly or with an intervening linker peptide sequence. The relocated N-terminal or C-terminal ends can be covalently joined to, or bound with another mol. such as a nucleic acid mol., a lipid, a carbohydrate, a second polypeptide, or a nanoparticle. The modified chaperonin variants can assemble into double-ringed chaperonin structures. Further, the chaperonin structures can organize into higher order structures such as nanofilaments or nanoarrays which can be used to produce nanodevices and nanocoatings. In particular, the invention provides modified variants of Sulfolobus shibatae chaperonin TF55β. The sequences of the S. shibatae chaperonin TF55β modified variants are provided.

L49 ANSWER 2 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB This invention provided a method of expressing poorly expressed proteins such as antibody fragments and membrane proteins by fusing with peptidylprolyl isomerase (PPIase) that acts as chaperonin. Mouse antibody scFVs or human serotonin receptor were conjugated at C-terminal of PPIase and expressed in E. coli. The PPIase in the chimeric protein was immobilized on the solid support through microlite, cyclosporin and juglone. The immobilized fusion protein provided in this invention can be used for screening agonists and antonists of the poorly expressed proteins.

L49 ANSWER 3 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB The structure of a chaperonin caging a substrate protein is not quite clear. We made engineered group II chaperonins fused with a guest protein and analyzed their structural and functional features. Thermococcus sp. KS-1 chaperonin α-subunit (TCP) which forms an eightfold sym. double-ring structure was used. Expression plasmids were constructed which carried two or four TCP genes ligated head to tail in phase and a target protein gene at the 3' end of the linked TCP genes. Electron microscopy showed that the expressed gene products with the mol. sizes of .apprx.120 kDa (di-TCP) and .apprx.230 kDa (tetra-TCP) formed double-ring complexes similar to those of wild-type TCP. The tetra-TCP retained ATPase activity and its thermostability was significantly higher than that of the wild type. A 260-kDa fusion protein of tetra-TCP and green fluorescent protein (GFP, 27 kDa) was able to form the double-ring complexes with green fluorescence. Image analyses indicated that the GFP moiety of tetra-TCP/GFP fusion protein was

accommodated in the central cavity, and tetra-TCP/GFP formed the closed-form similar to that crystallog. resolved in group II chaperonins. Furthermore, it was suggested that caging GFP expanded the cavity around the bottom. Using this tetra-TCP fusion strategy, two virus structural proteins (21-25 kDa) toxic to host cells or two antibody fragments (25-36 kDa) prone to aggregate were well expressed in the soluble fraction of Escherichia coli. These fusion products also assembled to double-ring complexes, suggesting encapsulation of the guest proteins. The antibody fragments liberated by site-specific protease digestion exhibited ligand-binding activities.

L49 ANSWER 4 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB A review on development of cCAP (chaperonin-caged protein expression) method using archaeal chaperonin derived from Thermococcus sp. KS-1, application of the cCAP method, structure and function of chaperone-like archaeal PPIase (peptidyl prolyl cis-trans isomerase) as a FK605-binding protein, and use of the PPIase for expression of soluble fused proteins which is otherwise mainly expressed as inclusion body.

L49 ANSWER 5 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - An immunogen (I) for inducing an immune response against a desired antigen protein, comprises a fused protein in which the full-length or a part of a desired antigen protein and a folding factor or its subunit are bonded together through one or more peptide bonds, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) producing (I), involves translating a fusion gene that encodes a full-length or a part of a desired antigen protein and a folding factor or its subunit; and (2) composition (II) for immunological use, formed by mixing (I) and an adjuvant.

BIOTECHNOLOGY - Preferred Immunogen: In (I), the folding factor is chaperonine, which comprises number of chaperonine subunits. One or all parts of the chaperonine subunits are mutually connected by peptide bonds. The desired antigen protein is connected to the N- or C-terminal of the chaperonine subunits. The antigen protein is connected among the chaperonine subunits. (I) has a protease cleavage site between the chaperonine subunits and the antigenic protein. The chaperonine subunits are derived from bacteria, preferably archaebacterium or from eukaryotes. The antigen protein is contained inside the chaperonine ring formed by the chaperonine subunits. The chaperonine ring comprises 5-10 chaperonine subunits. The folding factor includes chaperonine and PPIase. The PPIase is derived from Escherichia coli or archaebacterium. The antigenic protein is serotonin 5 HT1aR. The fusion protein comprises full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR.

ACTIVITY - Immunostimulant.

MECHANISM OF ACTION - None given.

USE - (I) or (II) is useful for producing an antibody specific to the antigen protein, which involves immunizing an animal (except human) using (I) or (II) and extracting the antibody from the animal (claimed).

ADVANTAGE - (I) prevents the antigen protein from quick degradation in the blood of an animal. (I) provides effective immune response against a desired antigenic protein and enables reliable production of an antibody against the desired antigenic protein.

EXAMPLE - Immunogen comprising Escherichia coli derived chaperonine GroEL and an antigenic protein was produced as follows. Vector capable of expressing the fusion protein of GroEL and antigenic protein was assemble. The antigenic protein is full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR. The vector was introduced into a suitable host and the fusion protein comprising 7 subunits of GroEL and recombinant serotonin 5 HT1aR receptor was produced. The stereostructure of the obtained fusion

protein was examined. The structure analysis showed that the chaperonine subunits formed a ring structure inside which the antigenic protein was contained. The serotonin 5 HT1aR receptor was protected by GroEL. The immunogenicity of the fusion protein (immunogen) was evaluated. The fusion protein was admixed with incomplete Freund's adjuvant and a composition for immunological use was obtained. The composition was administered (subcutaneously) to a rabbit and antibody titer against serotonin 5 HT1aR receptor was measured. The results showed that the immunogen was efficient in inducing immune response against serotonin 5 HT1aR receptor (antigenic protein). (59 pages)

L49 ANSWER 6 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) recombinant antibody from a fusion protein comprising heavy chain and/or light chain antibody connected to N and C terminal of chaperonin sub-unit (2-10) through peptide bond, involves expressing a fusion protein by translating chaperonin, heavy chain antibody and/or light chain antibody gene and producing antibody by cleaving heavy-chain and light-chain antibody from fusion protein.

BIOTECHNOLOGY - Preferred Method: In (M1), the fusion protein consist of two chaperonin rings comprising 5-10 chaperonin sub-units. A two-layer structure bonded by noncovalent bond through the ring surface is formed. The heavy chain and/or light chain antibody are stored inside the chaperonin ring. (M1) further involves producing the expression vector that incorporates the gene which encodes fusion protein of a chaperonin and heavy chain antibody or chaperonin and light chain antibody, introducing the expression vector into the host for expressing a chaperonin-heavy chain antibody fusion protein or chaperonin-light chain antibody fusion protein, cleaving the heavy or light chain antibody from the above fusion protein, producing an antibody from the heavy chain and light chain antibody obtained by the above process. (M1) further involves producing expression vector that incorporates the gene encoding a fusion protein of chaperonin, where the gene encodes fusion protein of chaperonin, heavy chain and light chain antibody, introducing the expression vector into the host for expressing a chaperonin -antibody heavy-chain fusion protein and a chaperonin -antibody light-chain fusion protein, cleaving the heavy chain and light chain antibody from the fusion protein, and processing the antibody from the obtained heavy chain and light chain antibody. (M1) further involves producing the expression vector that incorporates the gene which encodes the fusion protein of a chaperonin and heavy chain antibody, and the gene which encodes fusion protein of a chaperonin and an light chain antibody, introducing the expression vector into the host for expressing a chaperonin-antibody heavy-chain-antibody light-chain fusion protein, cleaving an antibody heavy-chain-antibody light-chain composite from the above obtained fusion protein, and processing the antibody from the above obtained composite. (M1) further involves producing an expression vector that integrated the gene which encodes fusion protein of a chaperonin and heavy chain antibody, and a chaperonin and a light chain antibody, where the fusion proteins are present in two groups that can coexist within the same host of different plasmids, introducing the expression vector into the host for expressing a chaperonin -antibody heavy-chain fusion protein and a chaperonin -antibody light-chain fusion protein, cleaving the heavy chain and light chain antibody from the above obtained fusion protein and processing the antibody from the heavy chain and light chain antibody. (M1) further involves producing an expression vector that integrated the gene which encodes fusion protein of a chaperonin and heavy chain antibody and a chaperonin and a light chain antibody, where the fusion proteins are present in two groups

that can coexist within the same host of different plasmids, introducing the expression vector into the host for expressing a chaperonin -antibody heavy-chain-antibody light-chain fusion protein, cleaving the heavy-chain-antibody light-chain-antibody composite from the above fusion protein and processing the antibody from the antibody heavy-chain-antibody light-chain composite. The ratio of chaperonin, heavy chain antibody or an light chain antibody gene is n:1 that is co-expressed in a host cell, where n is 1-9 and the above mentioned gene is incorporated in the vector for encoding the fusion protein. The vector incorporates the gene that encodes one piece of chaperonin having 2-4 connection subunit. The antigen specific for the antibody is added, where the antibody is cleaved out from an heavy-chain antibody, light-chain antibody or antibody heavy-chain-antibody light-chain composite. The chaperonin-antibody heavy-chain fusion protein, chaperonin-antibody light-chain fusion protein or a chaperonin-antibody heavy-chain-antibody light-chain fusion protein comprises a disconnection sequence with limited degradation type protease in connection unit (chaperonin sub-unit) of a chaperonin, the heavy chain antibody and/or an light chain antibody. (M1) is carried out in a non-cell translation system. The heavy chain and light chain antibody comprises non-human or human-type chimeric antibody heavy chain and light chain. The non-human or human chimeric heavy chain and light chain antibody has an unchanged region derived from human and a variable region derived from mammals other than a human.

USE - (M1) is useful for producing recombinant antibody using host (e.g., bacteria, yeast, animal cell, plant cell, insect cell, an animal organism, a plant organism or an insect organism) and chaperonin that originates from bacteria, archaebacterium or eukaryote (claimed).

ADVANTAGE - (M1) efficiently produces recombinant antibody using a host and easily purifies the antibody.

EXAMPLE - No relevant example is given.(23 pages)

L49 ANSWER 7 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:

NOVELTY - Sustained-release formulation (I) comprising a fusion protein which consists of bioactive protein or a low molecular medical agent coupled to conveyance protein (II) which is linked through the peptide bond to the chaperonin, where the bioactive protein or low molecular medical agent coupled to (II) is accommodated inside the chaperonin ring, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for a fused protein, comprising (II) of a low molecular medical agent connected through the peptide bond to a chaperonin which forms a two layer structure, in which the chaperonin ring consists of 14-18 chaperonin subunits connected through the ring surface by the non-covalent bond, where (II) of low molecular medical agent is accommodated inside the chaperonin ring.

BIOTECHNOLOGY - Preferred Formulation: In (I), the (II) coupled to low molecular medical agent or bioactive protein, is connected through the peptide bond to the N-terminal and/or C-terminal of a chaperonin subunit. The fusion protein has the amino acid sequences recognized by the protease for protein cleavage, in the chaperonin subunit connecting region of (II). The amino acid sequence recognized by the protease, is produced by the solid tumor or a specific organ. The chaperonin forms the two layer structure in which chaperonin ring consists of 5-10 chaperonin subunits connected through the ring surface by the non-covalent bond, thus forming a fibrous structure. The chaperonin is present in bacteria, archaebacterium or an eukaryote. The bioactive protein contains the full length heavy chain of an antibody, a light chain and Fv single stranded antibody (scFV antibody), or their partial protein having six or more residues. The bioactive protein is the full length cytokine or virus antigen, or their partial protein having more than six residues. (II) is the full length blood serum protein or its partial protein having six or more residues.

The low molecular medical agent is an antiinflammatory agent, an anticoagulant, an analgesic or a psychotropic agent. The fusion protein is obtained by translating the gene containing sequences of gene which encodes bioactive protein, or (II) of low molecular medical agent, and a gene which encodes chaperonin sub-unit. The gene which encodes bioactive protein or (II) of low molecular medical agent is a partial gene which codes cDNA or amino acid sequences of 6 or more amino acid residues.

USE - (I) is useful for sustained release of bioactive protein or a low molecular medical agent.

ADVANTAGE - (I) enables stable and efficient sustained release of bioactive protein or a low molecular medical agent. (I) improves efficacy of the drug.

EXAMPLE - Preparation of the fused protein of a chaperonin (beta) sub-unit (TCP (beta)) dimer and a hepatitis C virus core antigen (HCVc) was as follows: The TCP(beta) gene having a fully defined sequence of 9641 base pairs as given in the specification was cloned by PCR, using genome of Thermococcus KS-1 strain as a template. The obtained gene fragment containing TCP(beta) gene was inserted into T7 promoter of a plasmid, to obtain the expression vector pETD (TCP(beta))2. The DNA fragment of HCVc was obtained by PCR, using the primers. The obtained DNA fragment of HCVc was inserted into pETD(TCP(beta))2, to obtain the expression vector pETCSD-TII2HC. The pETCSD-TII2HC was introduced into Escherichia coli DE3 strain, which was cultivated in 2XYT culture medium containing 16 g of bactotrypton, 10 g of yeast extract, 15 g/l sodium chloride and 100 microg/ml of carbenicillin, at 30 degrees Centigrade for 24 hours. After 24 hours, the cultivated E.coli cells were ultrasonically rushed, and the supernatant was centrifuged, subjected to nickel chelate column, TSKgel superQ-5PW column, and to sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis. Thus, the fusion protein was obtained which was detected by rag turn blotting using the anti-HCV core antigen monoclonal antibody. The obtained fused protein and TCP(beta) dimer were incubated in the presence of 1 mM ethylenediaminetetraacetic acid-sodium (EDTA-Na), and dialyzed with respect to the 50 m M phosphate buffer (pH 7.0). The 300 microl of fused protein solution was treated with 0.5 mg/ml of 2.6 unit Prescission protease, incubated at 10 degrees Centigrade, and subjected to SDS-polyacrylamide electrophoresis. Thus, the HCVc was obtained. The results showed that the sustained release of HCVc from the fused protein. (11 pages)

L49 ANSWER 8 OF 14 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN

AB DERWENT ABSTRACT:

NOVELTY - A fusion protein (I) comprising chaperonin and protein having an enzyme activity are connected through the peptide bond, where the chaperonin is forming the 2 layer ring structure which contains 5-10 chaperonin sub-unit met in non-covalent bond through the ring surface and the protein having enzyme activity is stored inside the chaperonin ring, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for bioreactor using (I).

BIOTECHNOLOGY - Preferred Fused Protein: In (I), the chaperonin originates in a thermophilus archaebacterium or a hyperthermophilic property archaebacterium. The protein having an enzyme activity is connected to N terminal of chaperonin sub-unit and/or C terminal chaperonin sub-unit through the peptide bond. The protein having an enzyme activity forms the quaternary structure of a dimer or more with a form of a homo or hetero and each sub-unit is connected to the chaperonin sub-unit through the peptide bond, where the protein which has an enzyme activity is oxidoreductase, transferase, hydrolase, lyase, isomerase, ligase, or catalytic antibody.

USE - (I) is useful as immobilized enzyme bioreactor (claimed). (I) is also useful for an enzyme electrode.

ADVANTAGE - (I) is a stable enzyme with respect to heat, strong acid, strong base and organic solvent.

EXAMPLE - Heat-resisting property of a chaperonin fused protein was analyzed as follows. The expression vector pETDH (TCP beta)4 AbH which

contained the fused protein of chaperonin beta sub-unit TCP beta tetramer and (GFP) green fluorescent protein was introduced into Escherichia coli BL21 strain. The obtained recombinant cell was cultured at 30 degrees Centigrade for 24 hours in 2XY.T. culture medium (containing 16g bactotrypton, 10g yeast extract, 5 g/l sodium chloride, 100 approximatelyg/ml carbenicillin). Then microbial-cells extract was subjected to heat processing at 75 degrees Centigrade for 30 minutes. A supernatant liquid was recovered by centrifugation and applied to the nickel chelate sepharose column. The fraction adsorbed to the nickel chelate sepharose with the 50 mM Na-phosphate buffer containing 100-mM imidazole was sufficiently eluted after washing with the 50 mM Na-phosphate buffer containing 1-mM imidazole. After excluding an imidazole by dialysis, the fused protein of a TCP beta tetramer and GFP was purified with the anion exchange chromatography. The fluorescence intensity was measured before heat processing and after heat processing at 70 degrees Centigrade for 30 minutes. On comparative analysis, light-emission of GFP was decreasing sharply with heat processing. Thus it found that the fused protein of a TCP beta tetramer and GFP was extremely excellent in a heat-resisting property compared with GFP. (8 pages)

L49 ANSWER 9 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB It is intended to provide a complex of chaperonin and a target protein by which the target protein can be handled more easily, and a method of producing the same; and a method of stabilizing the target protein, a method of immobilizing the target protein, a method of analyzing the structure of the target protein, a sustained-release agent and a method of producing an antibody against the target protein, each using the chaperonin-target protein complex. The above-described chaperonin-target protein complex contains a fused protein in which an affinity tag is attached to chaperonin subunits via a peptide bond, and a target protein to which the affinity tag shows a specific affinity. Owing to the specific affinity, the target protein is bonded to the affinity tag, thus forming a chaperonin ring structure consisting of a plural number of chaperonin subunits. Using to the chaperonin-target protein complex, the target protein can be stabilized and surely immobilized on a support without causing any change in its stereostructure. Thus, IgG was incubated with a chaperonin β-subunit-protein A fusion protein produced in E. coli to obtain a chaperonin -IgG complex.

L49 ANSWER 10 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB This invention provides a method of increase the efficiency of fusion protein production by using C-terminal deficient chaperonin. The protein to be expressed was conjugated at C terminal of a chaperonin and expressed in E. coli. The chaperonins were from E. coli (C-terminal fragment containing Thr522 deficient) and Methanococcus thermolithotrophicus (C-terminal fragment containing Gly deficient deficient). The protein to be expressed was encapsulated in the chaperonin complex and purified by cleaving with protease. The method provided in this invention is specifically effective for preparation of receptors such as G protein-coupled receptors, ion-channels and tyrosine kinase receptors.

L49 ANSWER 11 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB The invention provided a method of enhancement of target protein expression. The target protein was expressed with chaperonins (1-20) as a fusion protein. The chaperonins form a two layer protein complex by non-covalent binding and the target protein was capsulized in the complex. The method provided in this method can be used for expression of the proteins which can not be expressed in conventional expression system.

L49 ANSWER 12 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB This invention provides an expression vector, a host, a fused protein, a

protein, a process for producing a fused protein and a process for producing a protein which prevent the formation of an inert and abnormal protein in producing a recombinant protein and thus make it possible to efficiently produce a target protein in the natural type (i.e., a soluble type) in a large amount Namely, an expression vector containing (a) a first coding region encoding a polypeptide having a mol. chaperon activity (PPIase, peptidyl-prolyl cis-trans isomerase) and at least one restriction enzyme site into which (b) a second coding region encoding a protein can be inserted. In the above expression vector, the first coding region is effectively linked to a promoter and the restriction enzyme site is located within the same reading frame as the first coding region and in the downstream of the first coding region. Alternatively, the restriction enzyme site is located so that the second coding region inserted is effectively linked to a promoter and the first coding region is located within the same reading frame as the second coding region and in the downstream of the second coding region. The vector provided in this invention can be used for production of proteins such as.

L49 ANSWER 13 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB Provided is a method for crystallization of any desired protein by recombinant expression as fusion protein with chaperonin subunit(s) for X-ray crystal structure anal. A target protein is either ligated to a chaperonin subunit via peptide bond or incorporated into the tertiary structure of the ring formed by chaperonin subunits. The target protein may be a membrane protein or nuclear hormone receptor. Expression of human cyclophilin (cyclosporin-binding protein hCyp) as fusion protein with Thermococcus chaperonin α subunit and expression of GFP as fusion protein with E. coli GroEL, and X-ray crystal structure study, are described.

L49 ANSWER 14 OF 14 HCAPLUS COPYRIGHT 2007 ACS on STN
AB Provided is an expression and cell-free translation system for a recombinant protein as fusion protein with chaperonin subunit. A target protein is incorporated into the tertiary structure of chaperonin as a fusion protein with a chaperonin subunit (namely, a mol. chaperonin of about 60 kDa, heat shock protein of 60 kDa or thermosome) to thereby inhibit the expression of toxicity of the target protein in the host,. Digestion with a protease or CNBr at a methionine residue can be used to cleave off the target protein from the chaperonin subunit. Bacteria, archaea, or eukaryote derived chaperonin is used. Bacteria, yeast, animal cell, plant cell, insect cell can be used as host. Mammalian antibody heavy chain, light chain, single chain antibody Fv region, virus antigen, transmembrane receptor, or cytokine may be the target protein. Expression of hepatitis virus B surface antigen, hepatitis virus C core antigen, mouse anti-chicken lysozyme single chain antibody, human antibody heavy chain constant region, human interferon α2b, or human serotonin receptor as fusion protein with chaperonin GroEL, is described.

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